

MARKED UP VERSION OF AMENDMENTSSpecification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 7, lines 3 through 7 with the below paragraph, marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Figure 7 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand, SEQ ID NO:11; non-coding strand, SEQ ID NO:64) encoding [(SEQ ID NO:11) and deduced amino acid sequence (SEQ ID NO:12) of] the mouse Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence, and the deduced amino acid sequence of the Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence (SEQ ID NO:12).

Replace the paragraph at page 7, lines 8 through 11 with the below paragraph, marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Figure 8 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand, SEQ ID NO: 13; non-coding strand, SEQ ID NO:65) encoding [(SEQ ID NO:13) and amino acid sequence (SEQ ID NO:8) of] the mouse GM607'CL antibody kappa light chain variable region, and the deduced amino acid sequence of the mouse GM607'CL antibody kappa light chain variable region (SEQ ID NO:8).

Replace the paragraph at page 7, lines 12 through 23 with the below paragraph, marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Figure 9 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand SEQ ID NO:14; non-coding strand, SEQ ID NO: 66) encoding the mouse Act-1 antibody heavy chain and signal peptide, and the deduced amino acid sequence of the mouse Act-1 antibody heavy chain variable region and heavy chain signal peptide (SEQ ID NO:15). The nucleotide sequence of the variable region is joined to a nucleotide sequence which encodes a deduced mouse Act-1 heavy chain signal peptide sequence, to yield a composite sequence [(SEQ ID NOS:14 and 15)]. (The identity of the primer which amplified the heavy chain region was deduced from the degenerate sequence, and an amino acid sequence for the signal peptide was derived from the primer, downstream sequence and sequences of other signal peptides. The signal peptide shown may not be identical to that of the Act-1 hybridoma.)

Replace the paragraph at page 7, lines 24 through 31 with the below paragraph, marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Figure 10 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand SEQ ID NO:16; non-coding strand, SEQ ID NO: 67) encoding the human 21/28'CL antibody heavy chain and signal peptide, and the deduced amino acid sequence of the human 21/28'CL antibody heavy chain variable region and heavy chain signal peptide (SEQ ID NO:17). The nucleotide sequence encoding the variable region is joined to a nucleotide sequence which encodes a signal peptide sequence derived from the V<sub>H</sub> of human antibody HG3'CL (Rechavi, G., *et al.*, *Proc. Natl. Acad. Sci., USA* 80:855-859 (1983)), to yield a composite sequence [(SEQ ID NOS:16 and 17)].

Replace Table 3 at pages 53-63 with the below Table 3, presented on pages iii-xiii and marked up by way of bracketing and underlining to show the changes relative to the previous version of the table.

-if-i-

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
1	1	FR1	D	D*	D	D	D		
2	2	-	V	V	I*	I	V	buried	Canonical AA for L1 loop (Δ1).
3	3	-	V	V	V*	V	V		
4	4	-	V	M	M	M	M	buried	Buried between L1 and L2. V=9/245, M=202/245 in mouse κ-II. M=42/45, V not seen in human κ-II. If binding is poor, consider changing this to Val in second version.
5	5	-	T	T*	T*	T	T		
6	6	-	Q	Q*	Q*	Q	Q		
7	7	-	T	T	S	S	S	surface	Distal to binding site (BS). T=164/245 in mouse κ-II. T=10/37, S=27/37 in human κ-II.
8	8	-	P	P	P*	P	P		
9	9	-	L	L	L*	L	L		

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
10	10	-	S	S	S*	S	S		
11	11	-	L	L	L*	L	L		
12	12	-	P	P	P	P	P		
13	13	-	V	V*	V*	V	V		
14	14	-	S	S	T*	T	T	surface	Distal to BS. S=151/248 in mouse κ-II. T alone (30/30) seen in human κ-II.
15	15	-	F	L	P	P	P	surface	Distal to BS. F=9/253 in mouse κ-II. F not seen in human κ-II. P=29/31 in human κ-II.
16	16	-	G	G*	G*	G	G		
17	17	-	D	D	E	E	E	surface	Distal to BS. E=18/30, D not seen in human κ-II.
18	18	-	Q	Q	P*	P	P	surface	Distal to BS and on a turn. P alone (31/31) seen in human κ-II.

-v-

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
19	19	-	V	A	A*	A	Δ	buried	Pointing into core, but standard mouse to human change. V=66/253, A=187/253 in mouse κ-II. A alone (30/30) seen in human κ-II.
20	20	-	S	S*	S*	S	S	S	
21	21	-	I	I*	I*	I	I	I	
22	22	-	S	S*	S*	S	S	S	
23	23	FRI	C	C*	C*	C	C	C	
24	24	CDR1	R	R	R	R	R	R	Canonical AA for L1 loop.
25	25	-	S	S*	S*	S	S	S	Canonical AA for L1 loop.
26	26	-	S	S*	S*	S	S	S	Canonical AA for L1 loop.
27	27	-	Q	Q	Q	Q	Q	Q	Canonical AA for L1 loop.
27A	28	-	S	S	S	S	S	S	Canonical AA for L1 loop.
27B	29	-	L	L	L*	L	L	L	Canonical AA for L1 loop.

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
27C	30	-	A	V	L	L	A		Canonical AA for L1 loop.
27D	31	-	K	H	H	H	K		Canonical AA for L1 loop.
27E	32	-	S	S	S	S	S		Canonical AA for L1 loop.
27F	-	-	-	X	-	-	-		
28	33	-	Y	N	D	N	Y		Canonical AA for L1 loop.
29	34	-	G	G*	G	G	G		Canonical AA for L1 loop.
30	35	-	N	N	N	Y	N		Canonical AA for L1 loop.
31	36	-	T	T*	N	N	T		Canonical AA for L1 loop.
32	37	-	Y	Y*	Y*	Y	Y		Canonical AA for L1 loop.
33	38	-	L	L*	L*	L	L		Canonical SS for L1 loop.
34	39	CDR1	S	E	N	D	S		Packing AA. Unusual (117/1365). A, H and N most commonly seen here.

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
35	40	FR2	W	W*	W*	W	W		
36	41	-	Y	Y	Y	Y	Y		Packing AA. Most common AA.
37	42	-	L	L*	L	L	L		
38	43	-	H	Q*	Q	Q	Q	buried	Packing AA. H is unusual (31/1312). Q is most common AA (1158/1312). H=6/225, Q=219/225 in mouse κ-II. Q=15/17, H not seen in human κ-II.
39	44	-	K	K	K	K	K	K	
40	45	-	P	P*	P	P	P	P	
41	46	-	G	G*	G*	G	G	G	
42	47	-	Q	Q*	Q	Q	Q	Q	
43	48	-	S	S*	S	S	S	S	
44	49	-	P	P*	P*	P	P	P	Packing AA. Most common AA.
45	50	-	Q	K	Q	Q	Q	Q	

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
46	51	-	L	L	L	L	L		Packing AA. Most common AA.
47	52	-	L	L*	L	L	L		
48	53	-	I	I*	I*	I	I		Canonical AA for L2 loop.
49	54	FR2	Y	Y	Y*	Y	Y		Canonical AA for L2 loop.
50	55	CDR2	G	K	L	L	G		Canonical AA for L2 loop.
51	56	-	I	V	V	G	I		Canonical AA for L2 loop.
52	57	-	S	S*	S*	S	S		Canonical AA for L2 loop.
53	58	-	N	N	N	N	N		
54	59	-	R	R	R*	R	R		
55	60	-	F	F	A	A	F		
56	61	CDR2	S	S*	S*	S	S		
57	62	FR3	G	G*	G*	G	G		
58	63	-	V	V*	V*	V	V		
59	64	-	P	P	P*	P	P		
60	65	-	D	D*	D	D	D		
61	66	-	R	R*	R	R	R		

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
62	67		F	F*		F	F		
63	68	-	S	S	S*	S	S		Canonical AA for L2 loop.
64	69	-	G	G*	G	G	G		
65	70	-	S	S*	S*	S	S		
66	71	-	G	G*	G*	G	G		
67	72	-	S	S*	S	S	S		
68	73	-	G	G*	G	G	G		
69	74	-	T	T*	T*	T	T		
70	75	-	D	D	D	D	D		
71	76	-	F	F*	F*	F	F		Canonical AA for L1 loop.
72	77	-	T	T*	T*	T	T		
73	78	-	L	L*	L*	L	L		
74	79	-	K	K	K	K	K		
75	80	-	I	I*	I*	I	I		
76	81	-	S	S	S	S	S		

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
77	82		T	R*	R	R		surface	Distal to BS. T=6/221, R=211/221 in mouse κ-II. R=11/12, T not seen in human κ-II.
78	83		I	V	V*	V	V	buried	Pointing into core, but standard mouse to human change. I=6/213, V=195/213 in mouse κ-II. V alone (12/12) seen in human κ-II.
79	84		K	E	E	E	E	surface	Distal to BS. K=20/215, E=191/215 in mouse κ-II. E=9/12, K not seen in human κ-II.
80	85		P	A*	A	A	A	surface	Distal to BS. P=6/183, A=175/183 in mouse κ-II. P=1/12, A=11/12 in human κ-II.

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
81	86	-	E	E*	E	E	E		
82	87	-	D	D*	D	D	D		
83	88	-	L	V*	V	V	V	half buried	Dital to BS. V alone (12/12) seen in human κ-II.
84	89	-	G	G*	G*	G	G		
85	90	-	M	V	V*	V	V		Distal to BS. M=6/212, V=196/212 in mouse κ-II. V alone (12/12) seen in human κ-II.
86	91	-	Y	Y*	Y*	Y	Y		
87	92	-	Y	Y	Y*	Y	Y		Packing AA. Most common AA.
88	93	FR3	C	C*	C*	C	C		
89	94	CDR3	L	F	M*	M	L		Packing AA. L is unusual (93/1238). Q is most common AA (654/1238).
90	95	-	Q	Q*	Q	Q	Q		Canonical AA for L3 loop.

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>l</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
91	96	-	G	G	A	A	G		Canonical for L3/Packing AA. 3 <sup>rd</sup> most common AA.
92	97	-	T	T	L	L	T		Canonical AA for L3 loop.
93	98	-	H	H	Q	Q	H		Canonical AA for L3 loop.
94	99	-	Q	V	X	T	Q		Canonical AA for L3 loop.
95	100	-	P	P*	P	P	P		Canonical AA for L3 loop.
	95A	-		P	R*		-		
	95B	-		-	-		-		
	95C	-		-	-		-		
	95D	-		-	-		-		
	95E	-		-	-		-		
	95F	-		-	-		-		
96	101	-	Y	Y	X	Q	Y		Packing AA. 2 <sup>nd</sup> most common AA.
97	102	CDR3	T	T*	T*	T	T		Canonical for L3.

Table 3 Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
98	103	FR4	F	F*	F	F	F		Packing A.A. Most common AA.
99	104	-	G	G*	G*	G	G		
100	105	-	G	G	Q	Q	Q	half buried	Distal to BS. Q=12/13, G=1/12 in human κ-II.
101	106	-	G	G*	G*	G	G		
102	107	-	T	T*	T*	T	T		
103	108	-	K	K*	K	K	K		
104	109	-	L	L*	V	V	V	half buried	Distal to BS. L=5/14, V=9/14 in human κ-II.
105	110	-	E	E*	E	E	E		
106	111	-	-	I	I*	I	I		
106A	-	-	-	-	-	-	-		
107	112	FR4	K	K*	K	K	K		

Replace Table 4 at pages 65-77 with the below Table 4, presented on pages xv-xxvii and marked up by way of bracketing and underlining to show the changes relative to the previous version of the table.

REMARKS

The brief descriptions of Figures 7 through 10 have been amended to recite the SEQ ID NOS of the nucleotide sequence of the non-coding strand of the double stranded nucleic acid presented in the figures. These non-coding strand sequences are included in the Substitute "Sequence Listing" filed herewith.

Table 3 and Table 4 have been amended to recite the SEQ ID NOS of the amino acid sequences presented therein. Support for the amendments is found, for example, at page 51, line 8 through page 52, line 7, and in the Sequence Listing.

The Substitute "Sequence Listing" filed herewith complies with the current sequence rules.

This Amendment adds no new matter.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By   
Robert H. Underwood  
Registration No. 45,170  
Telephone: (978) 341-0036  
Facsimile: (978) 341-0136

Concord, MA 01742-9133

Dated: July 29, 2002